

SN 10,017,643
Docket No. S-96,583
In Response to Office Action dated May 9, 2005

2

AMENDMENTS TO THE SPECIFICATION:

Please replace paragraph 1 under RELATED APPLICATIONS on page 1 with the following amended paragraph:

This application claims the benefit of U.S. provisional patent application S.N. 60/275,753 filed March 14, 2001; and is a continuation-in-part of U.S. patent application S.N. 09/769,612, filed January 23, 2001, now U.S. Patent 6,721,664, issued April 13, 2004, which in turn is a continuation-in-part of U.S. patent application S.N. 09/512,962, filed February 25, 2000, ~~now allowed~~, all incorporated by reference and made a part of the disclosure herein.

Please replace paragraph 3 on page 14, beginning at line 19, with the following amended paragraph:

In order to evaluate the range of applicability of map-likelihood phasing and the utility of iterative phase improvement with this technique, several tests were carried out with model data, where the quality of phasing could readily be assessed. Figures 1A-B and 2A-C illustrate the convergence properties of map-likelihood phasing as a function of percentage of the asymmetric unit that is occupied by disordered solvent. Model datasets were constructed based on the refined structure of dehalogenase enzyme from *Rhodococcus* as described in Terwilliger, *Acta Cryst. D56*, pp. 965-972 (2000). To simulate varying amounts of solvent, varying numbers of water molecules and C-terminal residues were left out of the phase calculations. This led to models with solvent content ranging from 31% (as in the actual crystals) to 73%. Starting phase sets with simulated errors were constructed and used along with the model amplitudes in map-likelihood phasing. In these simulations, a mask defining the solvent and protein regions was calculated from the atomic coordinates in the model, defining all points within 2.5 Å of an atom as being within the protein region. In each test, 20 cycles of phase calculation followed by figure-of-merit weighted map calculation were carried out. For each cycle, the mean true figure of merit, given by the cosine of the phase error $\langle \cos \Delta\phi \rangle$ is plotted.

SN 10,017,643
Docket No. S-96,583
In Response to Office Action dated May 9, 2005

3

Please replace paragraph 1 on page 15, beginning at line 5, with the following amended paragraph:

Figure 1A shows the effect of the percentage of the cell occupied by the macromolecule and by "solvent" (actually simply absence of protein in these simulations) on the phases obtained from map-likelihood phasing. The starting mean true figure of merit in each case was 0.32. For simulations with about 50% solvent or greater, each cycle of map-likelihood phasing resulted in phases that were at least as accurate as those in the previous cycle, with convergence essentially complete within 20 cycles. For those with 39% solvent, the phases became slightly worse with map-likelihood phasing compared to the starting phases, and for the case with 31% solvent, they were considerably worse.

Please replace paragraph 2 on page 18, beginning at line 25, with the following amended paragraph:

Figures 7A and 7B illustrates the overall quality of maps and bias ratios (as in Figure 5) for map-likelihood phasing with 31%, 47%, and 73% solvent and including varying amounts of prior phase information, ranging from zero weight on prior phases, to equal weighting of prior phases and map-likelihood phases. For the simulations with solvent content of 31% and 47%, the overall quality of the maps generally increases as expected with inclusion of prior phase information, with mean electron density at coordinates of atoms in the perfect model with 31% solvent increasing from 0.89 (zero prior phase information) to 1.09 (1% prior information). When equal weight is placed on the prior information, overall quality decreases slightly, indicating that the prior phase probability distributions may not be quite optimal. For the simulation with 73% solvent, inclusion of prior phase information had only a small, and generally negative, effect on the overall accuracy of phasing. This is presumably due to the very high amount of unbiased phase information in the map-likelihood function in this case of high solvent content.